

Host range testing and biology of *Abia sericea* (Cimbicidae), a candidate for biological control of invasive teasels (*Dipsacus* spp.) in North America

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Abstract

Invasive teasels (*Dipsacus* spp., Dipsacaceae) are widespread in the USA, being present in 43 states and listed as noxious in five. The cimbicid sawfly *Abia sericea* (Linné, 1767) is under evaluation as a potential agent for classical biological control of teasels. The host range, biology, and life history of this insect were studied under laboratory conditions and in common garden experiments from 2006–2010 at the Agricultural University of Plovdiv, Bulgaria in order to determine if this biocontrol candidate justified the expense of further testing under quarantine conditions in the USA. In the laboratory, potted plants from twelve plant species belonging to seven families were tested in choice tests of oviposition and feeding. Eggs were laid only on *D. laciniatus* and *D. fullonum* plants with only one exception, on *Valeriana officinalis*, although the larvae that hatched from the latter did not feed on that plant. Larval feeding was observed only on *D. laciniatus*, *Knautia arvensis*, and *Scabiosa ochroleuca*, all in the family Dipsacaceae, which has no species native to the New World nor any of economic importance. In common garden tests into which adults and third- and fourth-instar larvae were released in separate tests, eggs were laid and larvae fed only on *D. laciniatus*. The results of these experiments indicate that *A. sericea* has a narrow host range, most likely limited to *Dipsacus* species, and a few other Dipsacaceae and that further pre-release studies in a US quarantine are warranted.

Keywords

Biological control of weeds, host specificity, parthenogenesis, sawfly, Symphyta

Introduction

Fuller's teasel, *Dipsacus fullonum* L., and cutleaf teasel, *D. laciniatus* L., are both native to Europe and western Asia and have become invasive alien weeds in non-agricultural habitats in the United States, now present in 43 states, listed as noxious in five, and invasive in at least 12 more (Rector et al. 2006). Teasels are also present in four Canadian provinces and listed as noxious in Manitoba (Werner 1975, Province of Manitoba 2011). These invasive teasels were probably introduced as contaminated seed of cultivated teasel, *D. sativus* (L.) Honck., an obsolete crop plant formerly used in wool processing (Ryder 1994). All members of the family Dipsacaceae are native to the Old World (Verlaque 1985) and none are of agricultural importance (Bailey 2001); thus, classical biological control of invasive teasels in the New World using natural enemies imported from their native range is considered to be a worthwhile pursuit (Rector et al. 2006).

A handful of natural enemies have been recorded from the invaded range of teasels in North America although they appear to have little effect in limiting spread of teasel populations (Rector et al. 2006, Dugan and Rector 2007). Surveys of natural enemies from the native ranges of *D. fullonum* and *D. laciniatus* have yielded 102 insect species, as well as 27 fungi, four mites, one nematode, and two viruses (Rector et al. 2006, Rector and Petanović 2012). Candidate biological control agents under study to date include the eriophyid mite *Leipothrix dipsacivagus* Petanović et Rector, 2007, the leaf-mining fly *Chromatomyia ramosa* (Hendel, 1923), the flea beetle *Longitarsus strigicollis* Wollaston, 1864, and the sawfly *Abia sericea* (Linné, 1767) (Rector et al. 2008, Pečínar et al. 2009, 2011, Stoeva et al. 2011, BGR unpubl. data). Some fungi have also been identified as promising candidate agents (Rector et al. 2006).

The sawfly *Abia sericea* belongs to the family Cimbicidae (Hymenoptera: Symphyta), subfamily Abiinae, a group for which biological information is somewhat sparse (Liston and Späth 2006). In Europe approximately twelve species are listed; these have been divided into two groups according to their host-plant association (Savina and Liston 2009). All records for larval feeding by the *A. sericea* species group (Savina and Liston 2009) refer to a few genera in the family Dipsacaceae with the exception of *Fragaria* sp. (Rosaceae) (Konow 1901, Krishtal 1959) although this latter host record has been specifically tested and refuted (Rector et al. 2008). *Abia sericea* has been reported from across Europe, covering much of the known distributions of *D. fullonum* and *D. laciniatus* (Verlaque 1985, Taeger and Blank 2011). It is not known as an agricultural pest in Europe (Rector et al. 2008) and its presence is considered as an indicator of pristine natural habitat in parts of northern Europe where it is locally endangered (Savina and Liston 2009).

The purpose of this study was to investigate the biology and host range of *Abia sericea* in order to determine whether this candidate for biological control of invasive teasels merits the time and expense of undergoing a complete host-specificity assessment, including tests on rare and endangered North American plants, under quarantine conditions in the USA. Biological notes about *Abia sericea* that are pertinent to its potential importation into North America are also reported.

Methods

Host-specificity testing

A test plant species list was developed in consultation with the USDA-APHIS Technical Advisory Group for Biological Control Agents of Weeds, based mainly on phylogenetic relationships with the target weed but also including economically important species and rare or threatened plants in related families that are native to the invaded range of the target in North America (Wapshere 1974, APG II 2003, Briese and Walker 2008). This list includes 51 plant species from 24 genera in nine families. Since the family of the target weed, Dipsacaceae, is native only to the Old World and contains no species of great economic importance, the list consists largely of North American plant species from the most closely related families to the Dipsacaceae (viz. Adoxaceae, Caprifoliaceae, Valerianaceae). From this test plant list, thirteen species were chosen for this study for choice oviposition and larval feeding bioassays in the laboratory (Table 2). Nine of those species were included in subsequent common garden oviposition and feeding experiments (Table 3).

Of the plant species tested in these experiments, *Dipsacus laciniatus*, *D. fullonum*, *Knautia arvensis* L. (Coult.), *Scabiosa ochroleuca* L., and *Cephalaria transylvanica* (L.) Roemer & Schultes were grown in pots from field-collected seeds, whereas carrot (*Daucus carota* L.), lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleracea* L.), sunflower (*Helianthus annuus* L.) and *Valeriana officinalis* L., plants were grown in pots from commercially available seeds. Young *Sambucus ebulus* L. plants were dug from the field and potted, while *Viburnum lantana* L., and *Lonicera caprifolium* L. were obtained as potted plants from a commercial nursery that did not use insecticides.

For the laboratory bioassays, potted plants from the test species were arranged in plastic cages measuring 40 × 40 × 20 cm, with each cage containing one *Dipsacus laciniatus* rosette and up to seven plants of different non-target species. Individual female sawflies that were reared in the laboratory were released into each cage to oviposit. Number of eggs laid and feeding by hatched larvae were recorded. For the larval feeding experiment, two laboratory-reared larvae were placed on each non-target plant in similar cages containing one teasel rosette and seven non-target plants. Plants were arranged with leaves of adjacent plants touching each other to allow larvae to move freely between them. The laboratory experiments were carried out at 24±2°C, RH 60–70% and photoperiod of 16L:8D.

The common garden bioassays tested the same plant species as in the laboratory bioassays with the exception of the non-target Dipsacaceae species, which were not included. Test plants in the same developmental stages as in the laboratory bioassays were transplanted into a Latin square design with a distance of 70 cm between the plants within rows. A total of 78 field-collected third- and fourth-instar larvae were released in early June 2010 at a rate of 1–2 per test plant; larvae were not released onto teasel plants. Nine adult female sawflies were released into the test plot to lay eggs on test plants on 24 June 2010 and three more were released on 1 July 2010.

Adults were collected from the field and released on the day of collection after 6:00 p.m. Leaves of the test plants were inspected for eggs and larval feeding during the three days following each release.

Biological studies

Adult females were collected from the field from several sites in Bulgaria (Table 1) and released to oviposit in cages with *Dipsacus* plants to produce larvae for the laboratory colony and for larval feeding bioassays. The field-collected females were not used in the oviposition bioassays since their previous oviposition history was not known. Longevity, fecundity, and duration of the different stages were studied under laboratory conditions. The laboratory experiments were carried out at $24 \pm 2^\circ\text{C}$, RH 60–70% and photoperiod of 16L:8D.

For the oviposition studies one newly emerged adult female was released along with a newly emerged adult male into a plastic cage ($20 \times 20 \times 40$ cm) with a potted, laboratory grown *Dipsacus laciniatus* or *D. fullonum* plant in the rosette stage. Adult sex was easily determined by the presence on the male of a wide, black band down the center of the dorsal, posterior abdomen. There were 36 replicated cages each containing one pair of sawflies. After the death of a female sawfly the plants in the cages were carefully inspected for the presence of eggs using a magnifying glass and the number was recorded. The longevity of the females was recorded. The duration of the egg stage was measured as the number of days from the first observation of oviposition until the hatching of the first larvae in each cage.

The duration of the larval stage was studied by carefully placing two newly hatched larvae on the leaves of a young potted *Dipsacus* rosette in a cage ($20 \times 20 \times 40$ cm) using a small plastic spoon. The duration of the larval stage was defined as the number of days from hatching to pupation. The duration of the cocoon stage was studied under laboratory conditions in plastic containers (300 ml) covered with muslin. Cocoons of larvae made on the same day were placed together on a bed of senesced, crumbled teasel leaves in a container. Each container was checked daily beginning one week after setting up the experiment. The duration of the cocoon stage was defined as the number of days from the spinning of the cocoon until emergence of the adult. The amount of time from construction of the cocoon by the pre-pupal larva until pupation was not measured.

Table 1. Locations, in Bulgaria, of collection of *Abia sericea*. Life stage and purpose of collection are noted.

Location	GPS coordinates		Stage collected	Bioassays
Galabovo	42°01.49'N	24°42.28'E	Adult	Biological studies/colony establishment
Klokotnitsa	42°24.25'N	25°27.41'E	Adult	Biological studies/colony establishment
Lovech	43°04.12'N	24°44.09'E	Adult	Biological studies/colony establishment
Lozen	42°37.56'N	23°30.36'E	Adult	Biological studies/colony establishment
Pleven	43°24.25'N	24°28.79'E	Adult	Biological studies/colony establishment
Porojna	42°03.11'N	25°08.59'E	Larva	Common garden host-specificity experiment
Trud	42°12.29'N	24°45.57'E	Larva	Common garden host-specificity experiment

Field observations

During field collections of *A. sericea* adults or larvae from 2006–2010, notes were made on their behavior and other characteristics. In addition, sympatric plants that are on the test plant list or congeners of those plants were identified in the field and examined for the presence of adults, larvae, or eggs of the sawfly. These plants included *Knautia* spp., *Lonicera* spp., *Sambucus ebulus*, and *Scabiosa* spp.

Results

Host-specificity tests

In the laboratory oviposition host-specificity bioassays, eggs were laid only on *Dipsacus laciniatus* and *D. fullonum* plants with one exception: a single female that laid eggs on a *Valeriana officinalis* plant (Table 2). In one replicate, no eggs were laid on any plant. In laboratory larval feeding choice bioassays, *A. sericea* fed only on plants in the family Dipsacaceae. This included all *Dipsacus* plants in 71 replicates, five of 43 *Knautia arvensis* plants, and one of 18 *Scabiosa ochroleuca* plants (Table 2). None of 28 *Cephalaria transylvanica* plants were fed upon nor were any plant species outside of the family Dipsacaceae (Table 2). In the common garden larval feeding bioassay in which larvae were placed on all plants other than teasels, all larvae left their plants within one day, with 24 of them moving to teasel plants where they remained and fed on teasel foliage (Table 3). The fate of the other 54 larvae was unknown. No larval feeding was observed on any test plants other than *D. laciniatus* in this test. In the common garden oviposition test, eggs of the sawfly were found on two of the *D. laciniatus* plants and on no non-target plants (Table 3).

Table 2. Choice trials for oviposition of adults and feeding of *A. sericea* larvae under laboratory conditions.

Test plants		Oviposition			Larval feeding	
Family	Genus/species	No. of plants	No. of plants with eggs	Total no. of eggs laid	No. of plants	No. of plants fed on
Dipsacaceae	<i>Cephalaria transylvanica</i>	28	0	0	28	0
	<i>Dipsacus fullonum</i> / <i>Dipsacus laciniatus</i>	71	70	2769	71	71
	<i>Knautia arvensis</i>	43	0	0	43	5
	<i>Scabiosa ochroleuca</i>	18	0	0	18	1
Adoxaceae	<i>Sambucus ebulus</i>	28	0	0	28	0
	<i>Viburnum lantana</i>	8	0	0	8	0
Caprifoliaceae	<i>Lonicera caprifolium</i>	18	0	0	18	0
Valerianaceae	<i>Valeriana officinalis</i>	28	1	47	28	0
Apiaceae	<i>Daucus carota</i>	25	0	0	25	0
Asteraceae	<i>Lactuca sativa</i>	25	0	0	25	0
	<i>Helianthus annuus</i>	25	0	0	25	0
Brassicaceae	<i>Brassica oleracea</i>	25	0	0	25	0

Table 3. Choice trials for oviposition of adults and feeding of *A. sericea* larvae in a common garden experiment. The test plot consisted of nine plants of each test species.

Test plants		Oviposition		Larval feeding
Family	Genus/species	No. of plants with eggs	No of eggs laid	No. of plants fed on
Dipsacaceae	<i>Dipsacus laciniatus</i>	2	57	9
Adoxaceae	<i>Sambucus ebulus</i>	0	0	0
	<i>Viburnum lantana</i>	0	0	0
Caprifoliaceae	<i>Lonicera caprifolium</i>	0	0	0
Valerianaceae	<i>Valeriana officinalis</i>	0	0	0
Apiaceae	<i>Daucus carota</i>	0	0	0
Asteraceae	<i>Lactuca sativa</i>	0	0	0
	<i>Helianthus annuus</i>	0	0	0
Brassicaceae	<i>Brassica oleracea</i>	0	0	0

Biological studies and observations

The duration of the developmental stages of *Abia sericea* is presented in Table 4. Under laboratory conditions *A. sericea* spent approximately three weeks in both the larval (23.6 ± 3.2 d) and cocoon (20.3 ± 2.7 d) stages. By contrast, the adult female (4.7 ± 1.1 d) and egg (4.3 ± 0.6 d) stages combined for just over one week. Fecundity of unfertilized females averaged 86.1 ± 36.0 eggs with a minimum of 35 and a maximum of 194.

Abia sericea females typically lay their eggs into the leaf margin, in groups of two to seven, inserting the ovipositor under the epidermis of the leaf while straddling the leaf edge (i.e. with three legs each on the upper and lower leaf surfaces). The eggs are elongate and oval in shape. When freshly laid they appear greenish and are inconspicuous within the leaf but prior to hatching they turn beige or bronze-colored and seem to protrude more.

On teasel plants, neonate larvae begin eating immediately upon hatching, entirely consuming their egg cases in the course of feeding on the foliage. It was noted that in the case of eggs laid into *V. officinalis* leaves (Table 2), no feeding of any kind was observed by the larvae on this plant. Their empty egg cases remained after they vacated the *V. officinalis* plant to feed on an adjacent teasel plant in the same cage.

Larvae from a single clutch of *A. sericea* eggs hatch within one day of each other and begin to feed immediately, most frequently eating at the margins of the leaf on which they hatch or making small holes near the leaf margins. The larvae feed on the leaves of the rosette and of bolted teasel plants, chewing through the entire thickness of the leaves. Young larvae feed gregariously on all but the main veins of the leaves, while later instars feed individually. A detailed description of the morphology of the larval stadia of *A. sericea* is provided by Savina and Liston (2009).

When resting the larvae have a specific pose, with their abdomen coiled, usually on the lower side of the leaf. When disturbed, larvae typically drop to the ground where they can be difficult to spot in leaf litter, due to their broken coloration. When touched the larvae release a defensive liquid, a reaction known as “reflex bleeding” similar to that described by Savina and Liston (2009) for the larvae of *Abia fulgens* Zaddach, 1863.

Table 4. Duration of developmental stages and fecundity of *A. sericea* females under laboratory conditions. The egg-to-adult mean is the sum of the durations of the component life stages and is presented as an estimate since individual insects were not tracked from egg to adult.

Stage	Duration of stages (days)				
	Mean	SD	min	max	N
Egg stage	4.3	0.6	2	5	936
Larval stage	23.6	3.2	17	31	45
Pupal stage (cocoon)	20.3	2.7	15	26	24
From egg to adult	48.2				24
	Longevity (days)				
Unfertilized adult female	4.7	1.1	2	6	36
	Egg productivity (number/female)				
Unfertilized adult female	86.1	36.0	35	194	36

The larvae of *Abia sericea* pupate in double-layered, light brown to dark brown cocoons, similar to those described by Liston and Späth (2006) for the sawfly *A. nitens* (Linné, 1758). Under laboratory conditions pupation took place most frequently on the soil surface in the pots or at the base of or under the pots where the pre-pupal larvae had crawled. At emergence the adult sawfly cuts a circular opening in the cocoon.

Under laboratory conditions, adult females began laying eggs immediately after emergence without mating, despite the presence of males in the cages. Providing cotton soaked in a 5% sugar solution or inflorescences of dipsacaceous plants did not stimulate the adults to copulate. Laboratory-raised adult females in this experiment laid their eggs parthenogenetically and the progeny were all male. Adults of *A. sericea* were active during the day and rested at night.

Larvae of the sawfly collected from various locations in Bulgaria frequently displayed symptoms of viral infection, e.g. lethargy, cessation of feeding, shrinking and darkening of the body, excretion of viscous exudates by which they sometimes became glued by their anal segment to the floor of the experimental cage. A virus from the family Iridoviridae was isolated from larvae with these symptoms but it has not been identified further. Under laboratory conditions the rate of mortality was quite high when virus symptoms were present, particularly for younger larvae (data not shown). Parasitism by the endoparasitoid *Himerta defectiva* Gravenhorst (Hymenoptera: Ichneumonidae) was also observed. In 2008, six *H. defectiva* adults emerged from cocoons made in the laboratory by 34 *A. sericea* larvae collected in the vicinity of Parvomay, Bulgaria.

Field observations

Adult *A. sericea* begin to emerge in the field at the end of April and are most abundant from May to mid-July and in September. In August no adults were found in the field. In the field, *A. sericea* larvae feed most actively at dusk, and throughout cloudy or overcast days. During sunny weather, especially with high temperatures and intense light

the larvae tend to rest on the undersides of the leaves. Pupae in the field were observed on the soil surface under teasel rosettes or under the ground litter and plant debris. In fields where natural populations of *A. sericea* were collected from *Dipsacus*, neither eggs, larvae, nor adults of *A. sericea* were ever found on sympatric plants of *Knautia*, *Lonicera*, *Sambucus ebulus*, or *Scabiosa*.

Discussion

There are two possible explanations for the lone occurrence of *A. sericea* oviposition on *V. officinalis*. The female in question may have been stimulated to mistakenly oviposit on a non-target plant by the presence of the adjacent normal host plant, viz. *D. laciniatus*, in the same cage (Marohasy 1998). Indeed, the same female also laid more than 100 eggs on that teasel plant. Alternatively, this female may have been naturally stimulated to oviposit on *V. officinalis* although her offspring were apparently not stimulated to feed on that plant nor did they feed on their egg cases, as all other neonate *A. sericea* larvae in this study were observed to do. Instead, all of them moved to an adjacent teasel plant to feed. It is not known whether feeding on egg cases provides any nutrition or feeding stimulation to the neonate larva. Taken together, these data suggest that *V. officinalis* is not a genuine host for *A. sericea*. However, several North American *Valeriana* species, that are rare or endangered are included on the full test plant list and should be thoroughly tested in both choice and no-choice feeding and oviposition studies in subsequent pre-release evaluations.

It is not known whether *A. sericea* overwinters as a pupa or as an eonymph in its cocoon. Such data would require destructive sampling of the cocoons, which was not undertaken in this study due to the need for adults in the oviposition bioassays. Likewise, the point at which the larva becomes a pupa, during the three-week period within the cocoon, was not determined.

In the laboratory bioassays the males seemed disinterested in copulation. Savina and Liston (2009) reported an observed copulation in the field while the female was feeding from a flower. Parthenogenesis was observed in this study and was also reported for *Abia lonicerae* (Linné, 1758) (Kangas 1945) and *A. mutica* Thomson, 1871 (Kangas 1946). Further studies of adult reproductive behavior will be necessary to achieve successful copulation of *A. sericea* in the laboratory in order to facilitate mass-rearing for possible exportation and release for biological control purposes.

The observation of diurnal adult activity in this study was consistent with observations of other *Abia* species (Liston and Späth 2006, Savina and Liston 2009). The absence of adults in the field in the hottest part of the summer may be due to high temperatures and low humidity. It is notable that the adults reappear at the end of summer.

The larvae of the first (spring) generation, developing in May and June, and of the third (autumn) generation, developing in September and October, should have greater impact on *Dipsacus* populations than those of the second (summer) generation since the spring and fall generations have only rosettes to feed on, whereas the summer larvae can also feed on leaves of the bolting plants. Teasels are monocarpic perennials and

damage to rosettes may delay or prevent subsequent bolting, whereas damage to the large, bolting plant is unlikely to significantly reduce seed production.

Conclusions

The purpose of these experiments was to make a preliminary estimate of the host range of *A. sericea*. Regulators are charged with judging from this type of data whether an insect is likely to become a nuisance to non-target plants or other species, particularly those of economic or ecological importance, if the insect is imported and released for the purpose of controlling a targeted weed species. Depending on the perceived importance of a given non-target plant, any amount of feeding or other damage by a candidate biological control agent may be cause for concern, especially if the insect is able to complete its life-cycle on the non-target plant, regardless of marked preference for the target weed. Some may interpret such a result as an indication of sufficient genetic variability for host-acceptance in the tested insect that would ultimately allow it to include the non-target plant as a host. Under this interpretation, a new host association could be selected for over time after release of the insect into a new environment containing the non-target plant.

The results of the host-specificity tests reported here suggest that the tested *Abia sericea* populations from Bulgaria are highly host specific and likely to be restricted to host species from the family Dipsacaceae. While plants from other genera within this family have been recorded as hosts for populations of *A. sericea* from other parts of Europe (Savina and Liston 2009), the populations studied here fed mainly on *Dipsacus* species. Based on these results, more comprehensive host-specificity testing is warranted, including tests in US quarantine facilities on rare or endangered plants that are sympatric with invasive teasels in their North American range, particularly *Valeriana* species.

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